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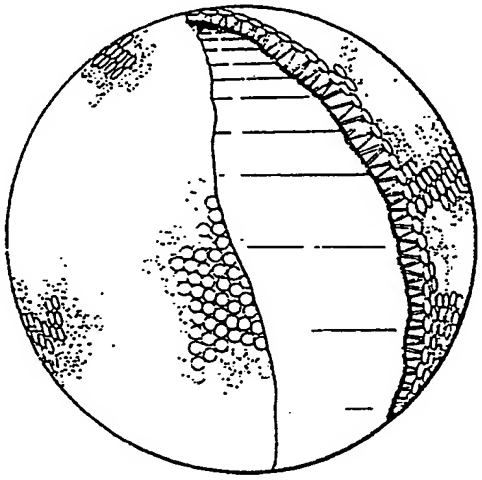

(57) Abstract

Microdroplets of water-insoluble drugs coated with a phospholipid are prepared by sonication. As an example, microdroplets of the general anesthetic methoxyfluorane coated by a unimolecular layer of dimyRistoyl phosphatidylcholine are prepared by sonication. The microdroplets so prepared remain stable in physiologically-compatible solution, and are suitable for injection, typically intradermal or intraveneously, into a patient for inducing local anesthesia. These methoxyfluorane-containing microdroplets have been demonstrated to cause long-term local anesthesia when injected intradermally, giving duration of anesthesia 28 times longer than with other anesthetics, such as lidocaine and 11 times longer than with bupivacaine. The latter is considered longest acting conventional local anesthetic. The microdroplet is also capable of solubilizing and delivering benzocaine and other water-insoluble drugs, and thus represents a novel drug delivery system and general method for delivery of water-insoluble drugs, lowering the necessary dose and providing a more direct and timed release.

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MICRODROPLETS OF WATER-INSOLUBLE DRUGS

BACKGROUND OF THE INVENTION

Microdroplets, originally called monolayer vesicles, were previously used to study the properties of the phospholipid surface as a model for the true phospholipid vesicle which, in turn, was a model for the biological membrane. This approach is to be distinguished from liposomes (multilamellar-) and unilamellar phospholipid vesicles used to deliver water-soluble drugs to the interior of cells, both in vivo and in vitro. These liposomes are true vesicles and consist of a spherical lipid bilayer with an aqueous phase inside.

Microdroplets are known and consist of spheres of organic liquid phase drug approximately 500 Angstroms in diameter and are covered with a monolayer of a suitable phospholipid.

The microdroplets of the invention can be used to deliver any water-insoluble/oil-soluble drug compound via injection. Most non-polar drugs now taken orally are contemplated and are within the scope of the invention. The organic liquid phase may be the drug itself, a general anesthetic medium, fluorocarbons, vegetable oil or mineral oil. The advantages of the microdroplets provided by the invention include a relatively slow release of the drug substance to the tissues and allow for a targeted delivery by intelligent choice of the site of injection with lowered metabolic degradation, first pass effects, and toxic side-effects in the liver and other organs.

Local anesthesia is conventionally accomplished by injection of water-soluble compounds into the site to be anesthetized. For efficacy the

drugs need both hydrophobic properties, to bind to and cross cell membranes, and hydrophilic properties, to dissolve in water and diffuse to the site of action. The duration of anesthesia is limited by the fairly rapid process of absorption of the injected anesthetic into the blood. The currently-used example of a long-acting local anesthetic is bupivacaine which gives anesthesia for a few hours in some applications. There is a considerable need for a local anesthetic of longer duration, preferably of significantly longer duration. Instances of the need for longer anesthetic duration include the control of post-operative pain, relief of chronic pain in cases of pinched nerves, back pain and other applications requiring long-term nerve conduction block and the like. Management of long-term pain is done by analgesics, such as aspirin and opiods, but these are often ineffective and sometimes give unwanted side-effects.

In contrast to local anesthesia is general anesthesia, which is accomplished by inhalation of anesthetic gases to produce unconsciousness. These include nitrous oxide, halothane, isofluorane, enfluorane and methoxyfluorane. The first-named example is a true gas; the others are volative fluoro-chloro-hydrocarbons which exist in liquid form. Liquid general anesthetics are water-insoluble and immiscible. They are volatized into the air which the patient breathes, they gain access to the circulation through the lungs and cause unconsciousness by binding to the nerve membranes in the brain.

The novelty of one embodiment of the invention lies in the fact that it uses general anesthetics as local anesthetics. According to a current popular conception of physicians and biomedical scientists the use of inhalation anesthetics as local anesthetics is

impossible. The textbooks and scientific papers deal with the local anesthetics and the general (often termed "volatile" and "inhalation" anesthetics) as separate classes of drug substances. According to contemporary thought this division is correct since the volatile anesthetics exist as oil-like liquids which are impossible to inject due to their low solubility in water -- injection as such would be unthinkable. Injection of a liquid phase of any of the volatile anesthetics would result in membrane delipidation, cellular damage and eventual tissue necrosis. Dilution of such agents in saline is not feasible because of their water-insolubility. Yet it is this low water-solubility and high solubility in the membrane phase which makes these agents effective blockers of nerve conduction in the brain (and elsewhere, but with less physiological consequence).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a perspective representation, partially broken away, of a microdroplet of the invention containing an organic liquid and drug substance surrounded by a unimolecular lecithin outer surface;

FIGURE 2 is a graph based on the results of Example 1 comparing the percent response of 1% lidocaine over a period of up to 200 minutes following injection;

FIGURE 3 is a graph reporting the response for Example 1 as the percent response of rats to a pain stimulus induced by the tail-clamp technique, as

a function of time after injection of microdroplets of methoxyfluorane;

FIGURE 4 is a graph also based on Example 1 reporting the initial response in percent against the dose of methoxyfluorane, in volume percent; and

FIGURE 5 is a graph also based on Example 1 reporting the time necessary for recovery of 50% response after the injection of microdroplets of methoxyfluorane against concentration of microdroplets, in volume percent.

The uniqueness of the invention is that a means of reducing this liquid oil-like phase to microscopic droplets, for instance approximately 500 Angstroms (estimated by calculation) in diameter is now available. Moreover, these microscopic droplets are stabilized against coalescence by a monolayer of phospholipid. Upon intradermal injection these microdroplets become entrapped in the interstitial space between cells and release their anesthetic in a slow and sustained manner. While not wishing to be bound to any particular theory or mode of operation, three possible mechanisms are postulated for this: anesthetic diffusion, vesicle-cell membrane collision and fusion; see the discussion below. This is in contrast to normal elimination kinetics of an injected drug in which the drug is eliminated in a "first order" manner giving rise to an exponential decrease in concentration. With the controlled and sustained release, the concentration of the drug in the nerve and neighboring tissue does not reach toxic concentrations. The rate of release can be controlled by the choice of anesthetic agent, based on vapor pressure

and membrane solubility, and to some extent by the choice of lipid.

One skilled in the art following the instructions provided herein will have no difficulty in empircally determining an optimum relationship between anesthetic agent or water-insoluble drug substance and compatible lipid coating. For the least exchangeable agents and most non-reactive lipids, the duration of effect will be governed by the time which it takes for the microdroplets to be cleared from the interstitial space and pass into the lymphatic system. The same principles are applicable to the use of lecithin-anesthetic microdroplet as a carrier for other water-insoluble drugs such as benzocaine, dantrolene and the like.

Local anesthesia requires delivery of the drug directly to the nerve membrane. This requires that the drug be able to bind to membranes and to traverse lipid membranes, i.e., cell membranes, and that it be water-soluble and thus able to cross the aqueous regions between cells in order to diffuse to the nerve membranes. These requirements have been fulfilled by designing local anesthetics, for example procaine and lidocaine, which have both non-polar and polar structural features. Their water-solubility results in limitation of the life-time (duration) of anesthetic effect since the local anesthetics diffuse to capillaries and are removed by the blood in the above-mentioned first order process. Theoretically, this problem could be circumvented by employing local anesthetics which are poorly soluble in water, e.g., benzocaine, but the problem then becomes the delivery of the anesthetic. Water-insoluble local anesthetics are not absorbed well through the skin and it is not

possible to inject them as one injects the water-soluble ones.

As mentioned above, general anesthetics are gases and volatile liquids which are inhaled to produce unconsciousness. They are poorly water-soluble compounds which enter the bloodstream by absorption in the lungs and which are carried through the bloodstream by binding to blood cells and proteins. They work on the central nervous system because it is most susceptible to their action, given this mode of delivery.

A microdroplet in accordance with the present invention is represented in perspective, partially broken away, in Figure 1, revealing a center containing the water-insoluble/organic phase containing the drug substance, surrounded by an outer unimolecular layer of lipid, such as lecithin. The properties of phospholipid membranes are described inter alia in my article concerning divalent cation-ligand interactions appearing in Metal-Ligand Interactions in Organic Chemistry and Biochemistry, Pullman and Goldblum, Part 2, pages 189-212, D. Reidel (1977).

One of the unique features of the invention lies in the use of volatile liquid general anesthetics to produce local anesthesia. Prior to this invention was not considered possible because it is not possible to inject an organic phase into the skin or other tissues without producing local damage due to dissolution of cell membranes and general derangement. Such a procedure would be literally unthinkable. The invention allows the injection of volatile general anesthetics without damage.

The key to accomplish the desired injection is to reduce the water-insoluble oil or anesthetic

(liquid) phase to microscopic dimensions, typically by sonication, and then coat the resulting structure with a layer of a lipid. Preferred are the phospholipids, which are natural constituents of biological membranes and as such are biologically compatible. A phospholipid is chosen which exhibits repulsive interaction with the cell membrances in the target tissue such that the microdroplet remains integral for the maximum time.

As mentioned above, it is believed that the microdroplet can transfer anesthetic to the tissue and nerves by three possible mechanisms: (a) solution, (b) collision/aggregation, or (c) fusion. Comparisons of anesthethic response plotted against time in hours after injection shown in Figure 3. From this and from Figures 4 and 5 it can be deduced that the release of the anesthetic from the microdroplets is slow and sustained. Figure 3 shows that the response rats to pain stimulus induced by tail clamping is decreased by injection of 0.5 ml of 6.7% methoxyfluorane microdroplets. The initial responsiveness (t=0-2^1/2 hrs) is also dose-dependent as shown in Figure 4. The half-time for recovery of responsiveness increases with increasing anesthetic concentration, reaching a maximum of approximately 70 hours at high concentrations as shown in Figure 5. The above are illustrative and demonstate effectiveness using three anesthetics, variable doses at a number of sites on the rat. Lidocaine was used as a control. Durations of lidocaine anesthesia were always less than 1/10th that of preparations in accordance with the invention.

While the research work leading to the recognition and completion of the present invention has been conceived primarily with anesthetics, and will in large part be illustrated and explained herein

on that basis,the invention is not so limited and includes similar systems employing water-insoluble organic drug substances included in the unique drug delivery systems and procedures of my invention.

Microdroplet preparation: The preferred method of preparing the microdroplets of the invention is by sonication with a probe sonicator. This is described in more detail below. Alternatively, microdroplets can be prepared in a bath sonicator. For small scale preparations a 1.0 cm diameter test tube is suspended, with use of a test-tube clamp, in a bath sonicator filled with water. The components of the microdroplet (organic phase, phospholipid, physiological saline, and drug to be included) are first grossly mixed by shaking, Vortex mixing, Polytron or other methods. The homogenized suspension is then introduced into the bath sonicator and sonicated for 1-2 hours. If the preparation is to be done on a large scale, it will be possible to omit the test tube and introduce the components of the microdroplet directly into the bath sonicator.

Microdroplets may also be produced by high intensity mechanical agitation. Useful methods include the Waring blender, the Polytron and high frequency shakers such as a commercial paint shaker.

An alternative method to consider is the solvent dilution method. The desired constituents of the microdroplets are dissolved at high concentration in ethanol or another oil- and water-miscible organic liquid. The ethanol solution is rapidly diluted into the physiological saline solution with vigorous mechanical agitation to insure rapid mixing. The ethanol dissolves in the aqueous phase while the other constituents cannot. The finely-dispersed

constituents spontaneously form microdroplets; the ethanol can be conveniently removed by dialysis.

Microdroplets can also be formed by a process similar to spray painting. The constituents of the microdroplets are suspended and sucked into intake of a commercial spray painter and the resulting spray bubbled through a saline solution to trap the microdroplets.

By judicious choice of methods and materials the diameter of the microdroplets is controlled between approximately 500 Angstroms to several micrometers by controlling the method, power and lipid to organic phase ratio. Increasing the power or the ratio tends to give smaller microdroplets. If natural or unsaturated lipids are used preparation is conducted in an atmosphere free from oxygen.

Selection of organic phases: Microdroplets according to the present invention are prepared from a wide variety of organic phases which, for convenience, may be considered by the following non-limiting types or categories:

1. Volatile inhalation anesthetics include methoxyfluorane as well as halothane, isofluorane and enfluorane.

2. Alkanes include heptane, the heptane microdroplets can incorporate benzocaine which is suitable to produce long-duration local anesthesia. Higher molecular weight alkanes will also be potent. Mineral oil as the organic phase is also of interest as it is able to carry large quantities of water-insoluble drugs. Solubility may be increased by inclusion of more polar organic compounds with the alkane phase.

3. Natural, plant-derived "oils" are also broadly contemplated, including olive oil and various vegetable oils. The "oils" are preferably screened toxicologically.

4. Ethers: Microdroplets have been made from dipropyl ether (3.4 mg/ml dimyristoyl lecithin, 6.5% n-dipropyl ether, ±4.1 mg/ml benzocaine) and dibutyl ether (5 mg/ml dimyristoyl lecithin, 7.0% n-dibutyl ether, ±4.1 mg/ml benzocaine). The dibutyl ether microdroplets and mixed dibutyl ether/chloroform microdroplets were found to have anesthetic potency. However, the anesthesia was of shorter duration (approximately 1/2 hour) possibly due to the greater water solubility of the dibutyl ether and chloroform which contributed to its more rapid removal. Longer-chain analogues could yield longer durations of activity.

5. Esters: Any long-chain or hydrophobic ester is contemplated particularly as a useful device for delivering "pro-drugs" which would be transformed into the active drug by hydrolysis by serum or cellular esterases.

6. Other organic substances which have been shown to be bio-compatible. These include by way of example silicone and high molecular weight fluorocarbons.

The organic phase selected will be fully compatible with the drug substance employed and pharmaceutically acceptable for product formulation/preparation purposes. As with all medical applications once the microdroplets are successfully prepared from a given organic phase and the selected drug substance incorporated therein, toxicological and efficacy screening is routine. Preferably the various

components from which the microdroplets are made are subjected to toxicological screening as well.

Lipids: Various lipids are also suitable for use in preparing the microdroplets of the present invention. Mixtures of two or more such lipids are useful to vary the surface properties and reactivity. All of the microdroplets in the working examples reported herein are made primarily from lecithin (phosphatidylcholine). This, together with sphingomyelin which is also contemplated, constitutes Class A. In Class B, are listed the phospholipids which can also be used to make microdroplets in the pure form, but which will react with calcium in the serum to give microdroplet aggregation or binding to cell membranes. The tendency to do this can be decreased by dilution with phosphatidylcholine, and thus there is a means of controlling the reactivity of the microdroplet. Class C contains only one representative, phosphatidylethanolamine. In the pure state it self-aggregates in a calcium-independent fashion. It is expected to have strong tendencies to aggregate with cell membranes. This tendency can be decreased by diluting it with lecithin. Class D, the steroids, do not form membranes or microdroplets by themselves, but which can be incorporated into the membrane, increasing its stability and decreasing its reactivity. Class E includes all molecules which can be accommodated in the monolayer. These are amphipathic molecules which can serve to change the nature of the monolayer surface and microdroplet reactivity.

CLASS A: Primary Lipids (usable in pure form):

Lecithin (phosphatidylcholine)
Sphingomyelin

CLASS B: These can be used in the pure form to make microdroplets (or phospholipid vesicles). They are expected to be highly reactive because of calcium-dependent aggregation. Preferably these lipids are mixed with lecithin to obtain controlled degrees of reactivity with cell membranes. Mixing in phospholipid vesicle preparations has already been demonstrated.)

Phosphatidic acid
Phosphatidyl serine
Phosphatidyl inositol
Cardiolipin (diphosphatidyl glycerol)
Phosphatidyl glycerol

CLASS C: Phosphatidyl ethanolamine This can be used to make microdroplets in the pure form at pH 9; they will self-aggregate when brought to pH 7. This has been shown to be feasible in phospholipid vesicle studies. Microdroplets made from phosphatidyl ethanolamine are expected to be very reactive with cell membranes. It is suggested that this lipid can be included with the normal lecithin to increase the reactivity to cell membranes.

CLASS D: Steroids: These should not be used in the pure form to make microdroplets but can be mixed with lecithin or other lipids to produce a surface which is less reactive, and presumably more stable.

Cholesterol (natural constituent of membranes)
Estrogens: Estirol, estrone, estradiol and diethylstilbestrol
Androgens: Androstenedione, testosterone (The microdroplet would also constitute a delivery system for estrogens and androgens.)

CLASS E: Semi-lipoidal molecules which can incorporate into the monolayer and change the surface activity of the microdroplet. These molecules could also be delivered to the nerve by the microdroplet.

Stearylamine or other long-chained alkyl amines which can be primary, secondary, tertiary or quaternary substituted. These give the microdroplet surface a positive charge and make them more reactive for the cell membranes. These compounds could also be delivered to the nerve.

Arachidonic acid or fatty acids. Could be incorporated into surface giving altered lipid packing and increased reactivity with cell membranes. The microdroplet is also a means of delivery of arachidonic acid for manipulations of prostaglandins.

CLASS F: Membrane-active agents

Nystatin, amphotericin B and gramicidin. These are surface-active antibiotics which have been shown to bind to the surfaces of phospholipid membranes and change their permeability. They are expected to change the reactivity of the microdroplet. The microdroplet is also a means of subcutaneous delivery of these antibiotics.

Several forms of lecithin are contemplated. For example lecithin is available as egg or bovine heart lecithin (natural) or in several synthetic varieties which differ in chain length. These include chain lengths ranging from 4 to 19 carbons (Supelco, Inc.). Dimyristoyl (14 carbons) and didodecanoyl (12 carbons) lecithin were used in the working examples (below). Didodecanoyl lecithin (12 carbons) may be considered more useful because the microdroplets will be more resistant to aggregation below room temperature. It is believed that lecithins with chain lengths in the biological range (10-18 carbons) are useful in various applications. Unsaturated lecithins (dioeoyl), dilinoeoyl; alpha-palmito, beta oleoyl; alpha palmitoyl beta linoleoyl and alpha oleoyl beta palmitoyl) are also available. Diarachidonyl lecithin (highly unsaturated and a prostaglandin precursor) is also available, as is alpha palmito beta myristoyl (mixed unsaturated chains) lecithin.

Phosphatidic acid is available from egg or as synthetic compounds (dimyristoyl, dipalmitoyl or distearoyl, Calbiochem). Bovine phosphatidyl serine is available (Supelco or Calbiochem).

Phosphatidyl inositol is available from plant (Supelco) or bovine (Calbiochem) sources. Cardiolipin is available (Supelco) from bovine or bacterial sources. Phosphatidyl glycerol is available from bacterial (Supelco) sources or as synthetic compounds (dimyristoyl or dipalmitoyl; Calbiochem).

Phosphatidyl ethanolamine is available as egg, bacterial, bovine, or plasmalogen (Supelco) or as synthetic compounds dioctadecanoyl and dioleoyl analogues and dihexadecyl, dilauryl, dimyristoyl and dipalmitoyl (Supelco and Calbiochem).

Drugs: The following is a list of drug substances which may be incorporated into the microdroplets of the invention. This list is presented for purposes of illustration only and is not to be considered as limiting.

1. The volatile anesthetics are described above. They include methoxyfluorane, isofluorane, enfluorane and halothane. Heptane was also shown to have anesthetic potency.

The following drugs will be incorporated primarily in the organic phase of the microdroplet. They are all uncharged, lipophilic water-insoluble drugs which have high oil solubility. In their applications, the organic phases of the microdroplets are made from the organic phase demonstrating the greatest drug solubility in macroscopic tests.

2. Water-insoluble local anesthetics. At a level of 2 mg/ml benzocaine can be incorporated into a 10% heptane microdroplet suspension (8.3 mg/ml dimyristoyl lecithin).

3. Dantrolene, a direct-acting muscle relaxant, is incorporated into methoxyfluorane microdroplets, heptane or mineral oil microdroplets. The resulting microdroplet suspension is injected around muscles and nerves for control of spasticity. This could circumvent the problem of hepatic toxicity seen with chronic oral administration of the drug.

4. The barbiturates (barbituric acid, pentothanl, phenobarbital, etc.) have been shown to block ganglionic transmission. The hypnotic/sedatives of the benzodiazepine class (diazepam, oxazipam, etc.) are presently used as muscle relaxants. These effects could be amplified by direct injection via microdroplets and the central nervous system effects obviated.

5. The microdroplet is believed to be an excellent means of direct and targeted administration of anti-inflammatory agents. Phenylbutazone can be administered at high concentration at the site of inflammation. The side-effects of nausea and vomiting, typically seen with oral administration, would be largely circumvented and much higher local doses could be used. Other anti-inflammatory or anti-arthritic agents which could be used include acetaminophen and colchicine.

6. Present evidence suggests that the rate of release of water-insoluble substances from the microdroplets to the blood stream will be slow if the microdroplets are injected intradermally or intramuscularly. This slow release is believed to be useful for the following classes of drugs:

(a) cardiovascularly active drugs: propranolol, labetalol, reserpine, nitroglycerin;

(b) hormones: estrogens, androgens, anabolic steroids in cancer chemotherapy;

(c) spironolactone (diuretic);

(d) coumarin (and other oral anti-coagulants);

(e) oil-soluble vitamins;

(f) prostaglandins and their analogues.

7. There are a number of drugs which are suitable for incorporation into microdroplets but the advantages of this treatment form with intradermal or intramuscular injection are not particularly apparent at present. These include: tricyclic anti-depressants, phenytoin (antiepileptic), and other centrally-acting agents.

All parts and percentages reported herein are by weight and all temperatures reported in degrees

Celsius, unless otherwise indicated. The compositions of the invention can comprise, consist essentially of or consist of the materials set forth and the process or method can comprise, consist essentially of or consist of the steps set forth with such materials.

DETAILED DESCRIPTION OF THE INVENTION

EXMAPLE 1

Anesthetic-containing lecithin-coated microdroplets are prepared by sonication in the following manner. Dimyristoyl phosphatidylcholine (41 mg) is added to a test tube and methoxyfluorane (0.2 ml) is pipetted in. The mixture is swirled in the tube at approximately 37°C and the lipid is observed to dissolve or be suspended to a limited extent. Next, sterile physiological saline (3.0 ml) is added and the tube is suspended under a Sonifier ^{R} Cell Disrupter, Model W185D (Heat System and Ultrasonics, Plainview, New York). The microtip is inserted and the sample is sonicated gently (power stage 2) for approximately one minute until the sample is dispersed. The oil, solid and aqueous phases are not distinguishable and gross homogeneity is obtained. The result appears as a milky single phase.

Next, the power is increased to stage 4 and the sample is sonicated for a total of approximately 5 minutes. The sonication temperature is between 30° and 45°C. The temperature can be controlled either by circulation of coolant around the sonication vessel or by interrupting the sonication periodically and allowing the sample to cool. The result of the sonication is a stable, homogenous suspension of lecithin-methoxyfluorane microdroplets. At the stated concentration, the suspension appears slightly cloudy

to the eye; turbidity decreases with increasing dilution of the sample in accordance with Beer's Law. Efficacy and microdroplet properties do not depend on the concentration at which the microdroplets were prepared, as observed from experiments carried out over a wide range of concentrations. The preparation is stable for several days when stored at 30°C. The preparation retains the smell of methoxy-fluorane indicating that that component is there and is releasable. Control experiments in which the lecithin is omitted from the medium failed to give microdroplets; phase separation was obtained immediately.

The efficacy of the preparation was tested with laboratory rats using a tail-clamp assay according to the method of Munson et al; [Munson, E.S, Hoffman, J.C. and DiFazio, C.A. "The Effects of Acute Hypothyroidism and Hyperthyroidism on Cyclopropane Requirement (MAC) in Rats" Anesthesiology 29: 1094-1098 (1968). The anesthetic preparation was injected into the tail and injections were distributed over four sites (0.5 ml total) such that a 3-4 cm long weal was obtained, encompassing all sides of the tail. Anesthesia was determined as being either present or absent from the response of the animal to clamping of the treated area with forceps as visually observed by squeeks or rapid movement. Untreated areas of the tail served as the control for the responsiveness of the animal to pain. As additional controls, some of the animals were injected with saline or sonicated lecithin without anesthetic agents. These controls showed uniformly no effect.

The efficacy of the microdroplet preparation was compared with that of 0.5 ml of 1% lidocaine (Figure 2) and bupivacaine in separate animals treated

and tested in parallel. At least four animals were assigned to each treatment and dosage group. They were tested immediately after treatment and at timed intervals thereafter until complete responsiveness was obtained in all animals.

With lidocaine, the animals were rendered 0% responsive. On the time scale presented, the effect wore off rapidly. After 2.5 hours the animals were 50% responsive and no measurable effect is observed after six hours. A similar experiment was carried out using 0.5% bupivacaine which is the longest acting local anesthetic in clinical use. A similar response was observed (data not shown), the animals became 50% responsive after 6.5 hours and there was no measurable effect after 8 hours.

The results are shown in Figure 3 which illustrates the responsiveness of the 12 animals to the pain stimulus for the lecithin-methoxyfluorane microdroplet (1.28% lecithin, 6.25% methoxyfluorane) and for 1% lidocaine. "Responsiveness" is averaged for all animals (100 = full pain response in all animals; 0% = no pain response in all animals). This Figure shows the responsiveness as a function of time after treatment. In the period of 1 to 2.5 hours after injection the animals were rendered 8% responsive to the pain stimulus. The effect persists during the times that the lidocaine effect had worn off (cf. Figure 2).

Half-responsiveness was observed 70 hours after injection. The effect slowly wears off, with 100% responsiveness observed after approximately 140 hours, i.e., about six days.

Figure 4 shows the dependence of the initial responsiveness as a function of the dose. Figure 5 shows that the half-time for return to 50%

responsiveness and shows a sigmoidal dependence on the dose of methoxyfluorane microdroplets, reaching a maximal half-time of 70 hours. Both the initial responsiveness effects and the half-time effects depend on the microdrop concentration in a graded manner consistent with the proposed mechanism of action: Large doses create large reservoirs of anesthetic within the tissue which must be removed before responsiveness to pain stimuli can be observed. Smaller doses can be used to create marginal anesthesia for a shorter time. In the latter case the injected dose of microdroplets does not have sufficient reservoir capacity to saturate the tissue. The maximal half-time for return of responsiveness of approximately 70 hours observed at maximal dose is believed to reflect the time that it takes the vesicles to be cleared from the tissue via the lymphatics.

EXAMPLE 2

Example 1 was repeated this time using 6.7% n-heptane as the anesthetic and similar results were obtained.

EXAMPLE 3

Example 1 was repeated this time using microdroplets with a 1:1 mixture of n-dibutyl ether and chloroform as the organic phase (6.7%) but the anesthesia was of short duration (1-2 hours). This correlates with the increased volatility and water solubility of these agents which give more rapid removal via the blood. The n-dibutyl ether chloroform microdroplets were shown to be effective in solubilizing benzocaine, but no increased efficacy of anesthesia was observed.

EXAMPLE 4

Lecithin coated methoxyfluorane microdroplets were injected into the hind leg muscles of the rat (2.0 ml total dose) and this resulted in immobilization of its hind quarters for one day. Controlled injections of lidocaine gave only short-duration immobilization (approximately two hours).

EXAMPLE 5

Microdroplets were prepared as described in Example 1 except that the organic pnase consisted of 6.7% mineral oil and the phospholipid monolayer consisted of didocecanoyl (dilauryl) lecithin (12.8 mg/ml). The microdroplets were found to be stable at 37°C in vitro for over a month. The microdroplets were injected into the tails of two rats and no toxic effects were observed. Local anesthesia was not observed, in accordance with expectations since mineral oil lacks anesthetic potency.

EXAMPLE 6

Microdroplets were prepared as described in Example 1 except that the organic phase consisted of 2.42% methoxyfluorane, 2.42% n-dibutyl ether and 1.8% mineral oil solubilizing 1.8 mg/ml benzocaine and the phospholipid monolayer consisted of didocecanoyl (dilauryl) lecithin (12.8 mg/ml). The microdroplets were found to be stable at 37°C in vitro for over a month. The microdroplets were injected into the tails of two rats and no toxic effects were observed. Local anesthesia was observed with kinetics similar to that given in Figures 4 and 5 for 2.4% methoxyfluorane.

I CLAIM:

1. A microdroplet consisting essentially of a substantially water-insoluble drug substance sphere surrounded by a unimolecular phospholipid layer.

2. The microdroplet of claim 1 in which the sphere also includes a compatible, pharmaceutically acceptable organic liquid.

3. A timed release drug delivery vehicle composed of microdroplets consisting essentially of a substantially water-insoluble drug substance stabilized against coalescence and surrounded by a unimolecular phospholipid layer.

4. An injectable pharmaceutical composition comprising the microdroplets of claim 1 together with a pharmaceutically acceptable injectable vehicle.

5. The microdroplet of claim 1 in which the drug substance is a local anesthetic.

6. A method of inducing local anesthesia in a subject in need of same comprising administering the microdroplets of claim 5 at or near the site at which local anesthesia is desired.

7. The microdroplet of claim 1 in which the drug substance is a general anesthetic in liquid form.

8. A method of inducing local anesthesia in a subject in need of same comprising administering the microdroplets of claim 7 at or near the site at which local anesthesia is desired.

9. A process for producing microdroplets consisting essentially of a substantially water-insoluble drug substance, a compatible, pharmaceutically-acceptable organic liquid and a surrounding unimolecular lipid layer that stabilizes the microdroplets against coalescence, said process comprising the steps of:

(1) preparing an homogenized suspension of the microdroplet components including the drug substance, organic liquid and phospholipid, and

(2) subjecting the homogenized suspension prepared in step (1) to sonification for a time to produce a cloudy, stable suspension of microdroplets.

10. The process of claim 9 in which the resulting microdroplets range from about 200 Angstroms to about 10 micrometers in diameter.

11. The process of claim 10 in which the resulting microdroplets are of the order to about 500 Angstroms in diameter.

12. A suspension of microdroplets produced by the process of claim 9.

13. The process of claim 9 in which the sonification of step (2) is conducted for about 1 to 2 hours.

14. The process of claim 9 in which the drug substance is a local or general anesthetic in liquid form.

15. The process of claim 9 in which a phospholipid forms the unimolecular layer.

FIG. 1

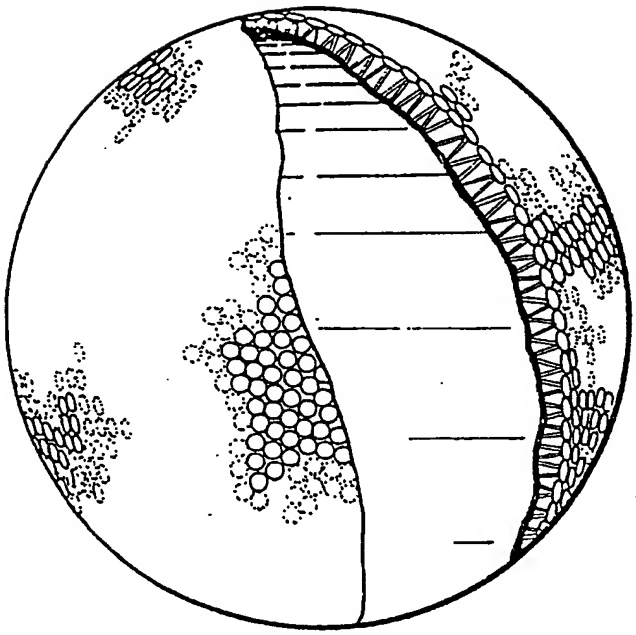


FIG. 2

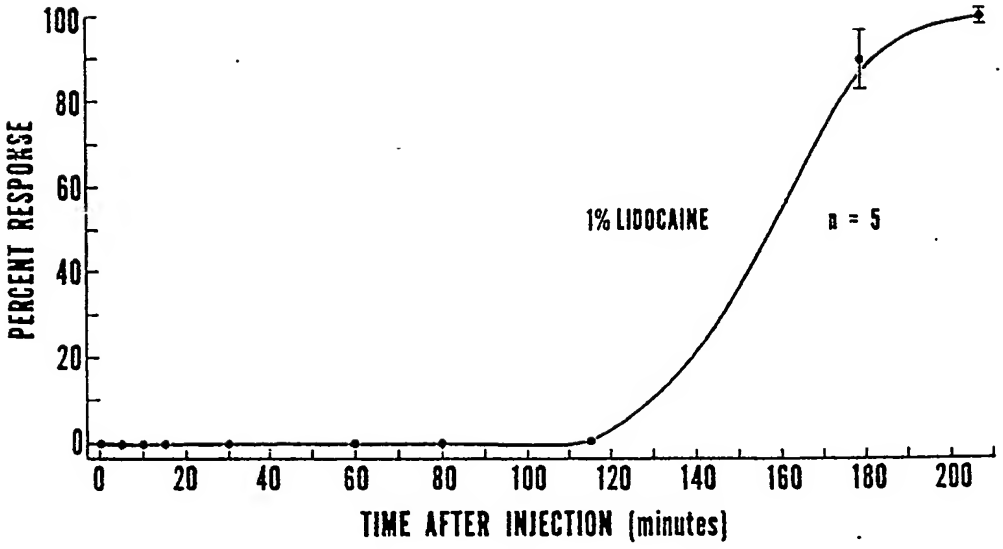


FIG.3

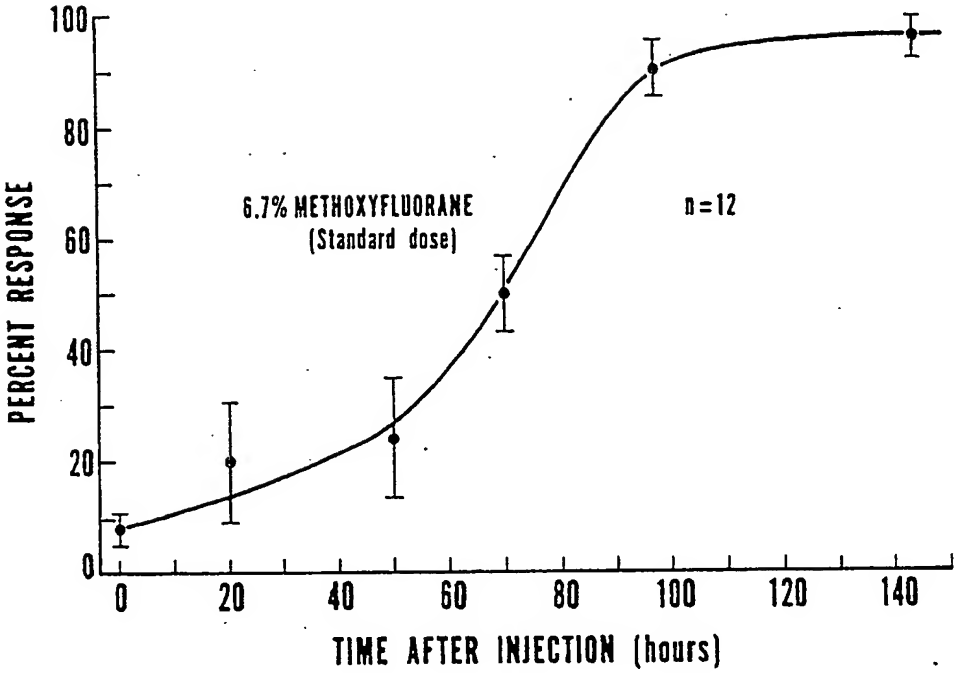


FIG.4

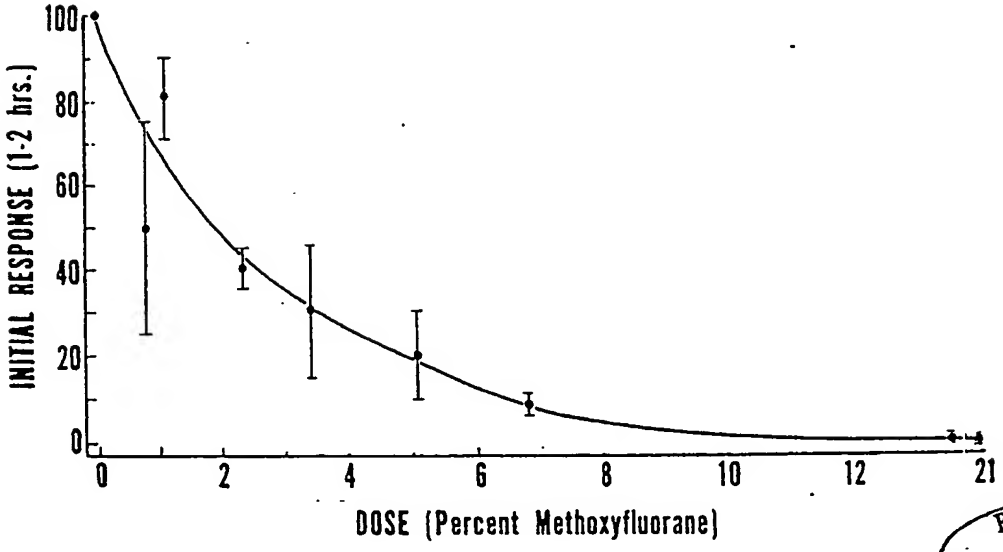
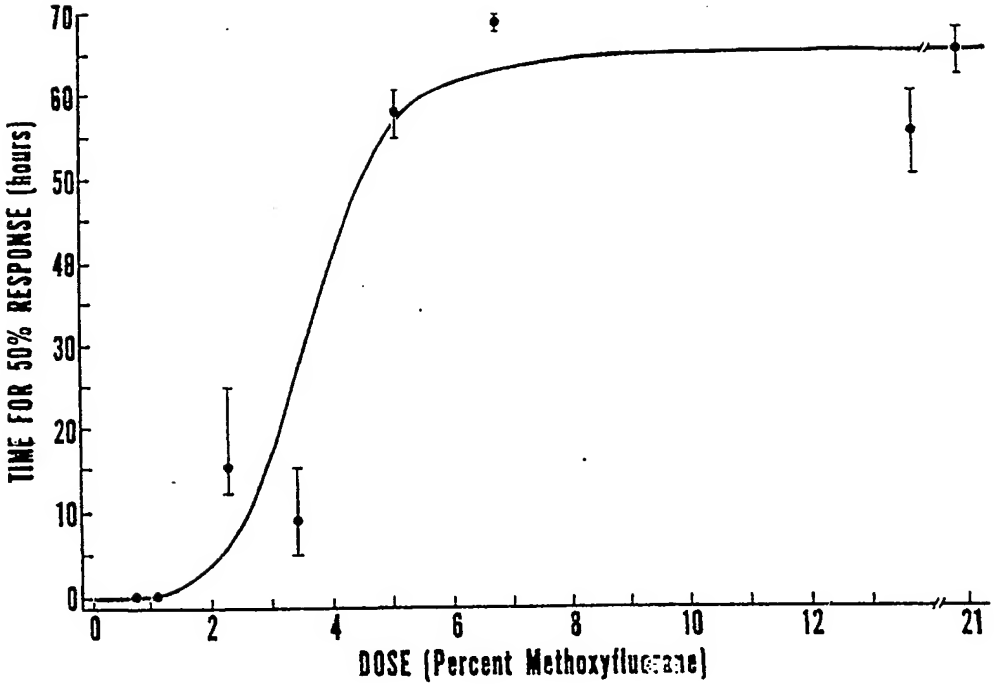


FIG.5



INTERNATIONAL SEARCH REPORT

International Application No PCT/US84/00906

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. Cl.³ A61K 9/22, 9/52, 9/42, 31/685, 47/00

U.S. Cl. 424/19, 38, 199, 365

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/19, 38, 199, 365 428/402.24

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 3,137,631, published 16 June 1964 Soloway et al.	
X,Y	US, A, 3,594,476, published 20 July 1971 Merrill	1-8
X,Y	US, A, 3,715,432, published 6 February 1973 Merrill	1-8
A	US, A, 3,755,557, published 28 August 1973 Jacobs	
A	US, A, 3,937,668, published 10 February 1976 Zolle	
X,Y	US, A, 4,145,410, published 20 March 1979 Sears	1-15
A	US, A, 4,147,767, published 3 April 1979 Yapel, Jr.	
A	US, A, 4,241,046, published 23 December 1980 Papahadjopoulos	1-15

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
23 August 1984	29 AUG 1984
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Ciro J. Faraci

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	US, A, 4,271,196, published 2 June 1981 Schmidt	1-8
Y	US, A, 4,302,459, published 24 November 1981 Steck et al.	1-8
Y	US, A, 4,308,166, published 29 December 1981 Marchette	1-15
X,Y	US, A, 4,320,121, published 16 March 1982 Sears	1-15

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☑ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☑ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
X,Y	US, A, 4,328,222, published 4 May 1982 Schmidt	1-8
Y	US, A, 4,331,654, published 25 May 1982 Morris	1-8
A	US, A, 4,351,831, published 28 September 1982 Growdon	
X,Y	US, A, 4,356,167, published 26 October 1982 Kelly	1-8
A	US, A, 4,369,182, published 18 January 1983 Ghyczy	
P,X,Y	US, A, 4,394,372, published 19 July 1983 Taylor	1-8
P,X,Y	US, A, 4,397,846, published 9 August 1983 Weiner et al.	1-15
P,Y	US, A, 4,448,765, published 15 May 1984 Ash et al.	1-15

See notes on accompanying sheet